SEPARATION OF THE ANTIBODY FRACTIONS IN HOG-CHOLERA SERUM *

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The study here reported was undertaken with a view to establishing the protective protein constituents in hog-cholera serum. It was not planned to work out the feature of concentration for practical therapy in hog-cholera at this time, much as it is desired. With the results given here, it is hoped that work will be done along the lines so well laid down by Gibson[1] and Banzhaf,[2] of the Department of Health of New York City, on the concentration of diphtheria antitoxin. They have demonstrated that artificial concentration is practicable. The procedure is based upon a fractionation of the constituent protein in order to determine the antibody fraction.

The literature on fractional precipitation of antibodies is not extensive. Pick[3] finds that diphtheria antitoxin is precipitated from goat's serum in the globulin fraction. Landsteiner,[4] studying the antitryptic action of blood serum, associates this property with the albumin fraction obtained after removal of the globulins by half saturation with ammonium sulphate. Opsonins in blood serum are said to be linked with the proteins separated by dialysis, as shown by Simon, Lamar, and Bispham.[5] They report that the euglobulin contains the opsonin, that the pseudoglobulin which remains in solution has none, and that the albumin is inactive. In a study on agglutinins by Gibson and Collins,[6] it is stated that the relative proportions of the agglutinins in polyagglutinative sera remain constant with regard to the proportional distribution of all of the agglutinins of the serum in the euglobulins and pseudoglobulins. They conclude from these results that it is not trustworthy to differentiate the antibodies contained in the euglobulin and pseudoglobulin fractions. A somewhat later work by Banzhaf and Gibson[7] gives results which show conclusively that the saturated sodium chlorid soluble serum gobulins of the higher fractions are more potent, per gram of protein, in antitoxin than those precipitated by lower concentrations of ammonium sulphate.

The concentration limits at which the several protein fractions will separate by addition of ammonium sulphate differ for various authors. Oppenheimer[8]

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1. Jour. Biol. Chem., 1905-1906, 1, p. 161.
2. Ibid., 1907, 3, p. 253.
3. Hofmeister's Beiträge, 1901, 1, p. 351.
4. Centralbl. f. Bakteriol., I, O., 1900, 27, p. 357.
5. Jour. of Exper. Med., 1906, 8, p. 651.
6. Jour. Biol. Chem., 1907, 3, p. 233.
7. Ibid., p. 253.
8. Grundzüge der Biochemie, 1909, p. 174.

places the limits of precipitation at 25 percent saturation for fibrin-globulin, 25-32 percent for euglobulin, and 32-48 percent for pseudoglobulin. According to Hofmeister and Pick,[9] one portion of the globulin, the eu-fraction, insoluble in water, corresponds to a precipitate obtained by 28-36 percent saturation with a saturated solution of ammonium sulphate; another part, insoluble in water, the pseudoglobulin, is precipitated by 36-44 percent saturation. Again, we find that seroglobulins can be separated by ammonium sulphate into three fractions, the limits of which are 28-36 percent., 33-42 percent, and 40-46 percent, all of them containing globulin insoluble in water (Porges and Spiro[10]). Haslam[11] splits serum protein into three bodies by means of ammonium sulphate. Globulin, water-insoluble, will come down with one-third saturation, pseudo-globulin with one-half saturation, and albumin will be soluble in a half-saturated solution of the salt. It is evident that differentiation on the basis of water solubility is untenable for globulin fractions. Results are at variance because of the readiness with which a soluble part becomes insoluble and vice versa. As noted in this study, phenol has a marked effect upon the ease with which the protein goes into solution. Particularly with albumin, ordinarily soluble in water, has this difficulty been encountered. It has been shown by Hammarsten[12] that exposure to the air and holding under water will affect solubilities of globulins. In general, it may be stated that the character of globulin is such that acid will favor its precipitation and lower the precipitation limits, as has been noted by Cohnheim.[13]

The separation of serum proteins is linked with great difficulties. There is always a tendency on the part of a residue of one fraction to remain dissolved in the other. Haslam[14] is of the opinion that proteins exhibit strong affinity for each other, and that a series of precipitations is necessary before a relatively purified fraction may be obtained. In an earlier work, this investigator pointed out that the solubility of globulins is largely increased in the presence of serum albumin and other serum constituents, so that, in an albumin filtrate, considerable globulin may be present. By means of a series of precipitations along fractional lines, he has been able to demonstrate the presence of a protein precipitate in a filtrate supposedly free from other fractions.

EXPERIMENTAL

The chief purpose of this study does not warrant the use of an extremely delicate method of separation of protein fractions since the extent of "contamination" would not affect the specific reaction of the animals to the possible protective property of the fraction. An attempt was made however to arrive at "pure" fractions in so far as this was

9. Hofmeister's Beiträge, 1901, 1, p. 351.
10. Cited by Hammarsten, Textbook of Physiological Chemistry, 1912, p. 251.
11. Proc. Physiol. Soc., Jour. of Physiol., 1912, 44, p. 241.
12. Ergebn. d. Physiol., 1902, 1, p. 330.
13. Chemie der Eiweisskörper, 1911, p. 180.
14. Jour. of Physiol., 1905, 32, p. 268.

possible. The results tend to show that it is possible to obtain pseudoglobulin practically free from euglobulin. This conclusion is based on the delicacy of the phosphorus test made on both fractions, one of which is known to be free of phosphorus (pseudoglobulin).

The solution of pseudoglobulin was well shaken and about 2 c.c. evaporated to dryness in a porcelain crucible. To the residue were added a small piece of solid sodium hydroxid and a few crystals of potassium nitrate, and the whole fused until a clear, white mass was obtained. After it had cooled, the crucible was half filled with distilled water and made acid to excess with a 10 percent solution of nitric acid. To a portion of this mixture, an equal volume of ammonium molybdate was added and the resultant mixture warmed. No yellow coloration or yellow precipitate was obtained. Euglobulin solution, treated similarly, gave a decided, finely divided, yellow precipitate, which settled on standing. A control test to determine the possibility of phenol interference was made with phenolized phosphate solution, and a positive result obtained. An attempt was made to test the purity of the fractions by means of precipitating serum, obtained with the respective fractions by injection into guinea-pigs. It was not possible to obtain a precipitating serum for the homologous protein. Further tests on rabbits might solve this point.

A preliminary qualitative test was made on one liter of old "serum" preserved with 0.5 percent phenol. One portion (350 c.c.) was filtered through asbestos wool under one atmosphere pressure, and 250 c.c. of the serum were fractionated at 25, 32, and 48 with saturated ammonium sulphate. The 25 percent fraction was too slight for detection. A fair precipitate was obtained with 32 percent saturation of the filtrate, and the filtrate from this fraction when made up to 48 percent yielded a heavy precipitate.

The precipitates of each fraction were separately dissolved in salt solution and the filter paper removed by straining through gauze. To each of these solutions, an equal volume of saturated ammonium sulphate was added and the fractions thus precipitated. The 32 percent fraction came down slowly. It appeared colloidal at first and, after two or three hours standing, flaked out satisfactorily. The precipitates were next treated with a volume of saturated sodium chlorid solution equal to twice that of the original volume of the serum used (i. e., 500 c.c.). The filter paper was strained through gauze and the extract allowed to stand twenty-four hours. Addition of saturated ammonium sulphate gave no precipitate. Repeated trials gave the same result. The apparently insoluble residue was dissolved in salt solution and easily precipitated with an equal amount of saturated ammonium sulphate. The explanation for this is not apparent and it seems surprising in view of the fact that Gibson[15] was able to dissolve his antitoxic globulins in saturated sodium chlorid solution. It may be that the hog-cholera antiserum possesses a different chemical constitution, or that the phenol used as a preservative exerts an untoward influence over the inherent solubilities.

A second portion of this lot of serum was not filtered and was treated in the same manner to separate the protein fractions. It was found that filtration of the precipitates was retarded because of the presence of blood corpuscles and suspended corpuscular material. From a quantitative viewpoint, it would appear that the corpuscles introduce an error in the determination of the individual fractions since the primary precipitate would be unduly augmented by the

15. Jour. Biol. Chem., 1905-1906, 1, p. 161.

mechanical inclusion of the corpuscles. On the other hand, if the corpuscles are disrupted or dissolved, as was the case in the phenolized test serum used, the error would be slight. The method of separation just outlined was discarded in favor of the one to be subsequently presented.

The serum used for fractionating was obtained from hogs which had been immunized by means of a single injection of virus in a dose of 5 c.c. per pound body weight. The animals which showed the best temperature curve and best physical condition were bled from the tail after about ten days. The blood was collected with the usual precautions to insure sterility and was preserved with 0.5 percent phenol. The virus used for hyperimmunization was taken from such animals as showed the most characteristic lesions of hog-cholera. The antiserum was pooled from a number of hogs. This lot, known as Serial 121, was used in this work. The potency of the serum was tested by injecting, intramuscularly, varying amounts plus the virus. The test was begun Oct. 3, 1914. Animals were off test Oct. 24, 1914. The report is given below:

No. of Hog	Weight in lbs.	Virus in c.c.	Serum in c.c.	Temperature											
				10/5	10/7	10/8	10/9	10/10	10/12	10/13	10/14	10/15	10/16	10/17	10/20
1	80	2	25	102.4	102.8	102.4	103.2	103.2	102.6	102.8	102.2	102.6	102.4	103.4	102.5
2	60	2	20	102.2	102.2	102.6	104.4	104.6	103.5	103.8	102.6	103.2	103.0	102.4	103.0
3	50	2	15	102.4	102.8	102.5	107.6	107.1	106.4	108.8	105.0	102.8	102.4	103.2	103.4
4	85	2	25	102.0	102.4	101.9	103.4	104.8	104.2	103.8	103.2	103.2	103.6	102.6	101.2
5	60	2	20	101.8	101.6	102.8	103.6	103.8	102.6	103.0	102.4	103.0	103.2	103.8	102.4
6	75	2	25	102.2	102.4	102.0	103.8	104.2	103.2	103.6	103.0	102.4	102.8	102.4	104.0
7	70	2	0	102.6	102.4	102.2	105.8	106.8	Moribund, killed						
8	50	2	0	102.0	102.2	101.8	106.2	106.6	Moribund, killed						
9	85	2	0	102.4	102.6	103.2	105.6	106.4	Moribund, killed						
10	75	2	0	102.2	102.0	102.6	106.0	106.6	Moribund, killed						

From these figures, we note that 15-25 c.c. of the serum gave protection against 2 c.c. of virus. As, with one exception (Hog 3), the animals showed but slight fluctuations in temperature, it is evident that smaller amounts of serum would have sufficed to carry the animals through.

Test A.—Five hundred cubic centimeters of serum were filtered through asbestos wool under one atmosphere pressure. Two hundred and fifty cubic centimeters of the antiserum were used in the chemical separation, 100 c.c. kept at room temperature, and 100 c.c. preserved for quantitative analysis. Another lot of serum from the same stock was not filtered and was fractionated in the same manner (to be described).

In this connection might be mentioned the question of the value of red blood cells in immunization with hog-cholera serum. It was the original plan of this study to test both filtered and unfiltered serum freshly drawn from immune hogs with this end in view. At about this time, a paper appeared by Haslam and Franklin[16] in which they show that the blood cells exert no protective value whatever. The authors conclude moreover that a serum from which red blood corpuscles have been removed, is definitely more potent than one containing them.

16. Jour. Infect. Dis., 1914, 14, p. 257.

This conclusion, however, is not warranted, since injections were made with whole blood (termed "whole serum" by the writers) and centrifugated blood, no account having been taken of the actual serum volume present in each. The writers state that 0.3 c.c. of "whole serum" protected animals from hog-cholera, whereas 0.2 c.c. of the centrifugated serum were sufficient. If, as they point out, the corpuscles are inactive and comprise 40 percent of the volume of the "whole serum," then there are in reality but 0.18 c.c. of serum actually present in the amount injected. What has been shown then is merely that an added bulk of corpuscles may be dispensed with, for if the centrifugated material were more potent, less than 0.18 c.c. of serum would be necessary for a protective dose. In future work, it might be advisable to adopt uniform terms for the material injected. As used today, the so-called hog-cholera serum is in fact whole blood. Centrifugated blood or serum from clotted whole blood might be termed serum more accurately.

The method of fractionation used was as follows: 250 c.c. of the defibrinated hemolyzed blood (called "serum") (121) were precipitated with an equal volume of saturated ammonium sulphate, allowed to stand one hour (throughout the experiment the period of time allowed to elapse before filtering) and filtered. The precipitate was washed with about 100 c.c. of 50 percent ammonium sulphate solution (amount used for washing of all precipitates), pressed almost dry between filter papers, and then dissolved in approximately 300 c.c. of physiologic salt solution. This was again precipitated with the necessary amount of saturated ammonium sulphate, washed with 50 percent ammonium sulphate, and dried between filter papers. The filtrates were added to the first albumin filtrate. The globulin precipitate was dissolved in about 300 c.c. of physiologic salt solution, precipitated with a volume of saturated ammonium sulphate necessary to bring the concentration to 33⅓ percent, and filtered (Euglobulin 1). The filtrate containing the pseudoglobulin fraction was measured and precipitated with a volume of saturated ammonium sulphate sufficient to bring the concentration to 50 per cent (see note). The solution was filtered and the precipitate (Pseudoglobulin 1) was dissolved in physiologic salt solution. To this was added saturated ammonium sulphate to 33⅓ percent concentration. A very small precipitate was obtained. The solution was poured through the filter containing the main euglobulin precipitate. The usual volume of 33⅓ percent ammonium sulphate was used in washing, and this was added to the pseudoglobulin filtrate, which was in turn brought up to 50 percent concentration with saturated ammonium sulphate, allowed to stand, and filtered (Pseudoglobulin 2). The filtrate containing albumin was added to the original albumin filtrate. The total euglobulin precipitate, pressed dry between filter papers, was dissolved in about 300 c.c. of physiologic salt solution, precipitated by one-third saturation with ammonium sulphate, and filtered (Pseudoglobulin filtrate 2). The precipitate (Euglobulin 2) was washed and the washings added to the filtrate which was freed from pseudoglobulin by bringing up the ammonium sulphate concentration to 50 percent with addition of a saturated solution. This precipitate

(Pseudoglobulin) was added to the main precipitate and the filtrate combined with the total albumin filtrates. The albumin was separated by addition of a small amount (3 c.c. per liter) of glacial acetic acid and ammonium sulphate crystals to complete saturation at room temperature. This was allowed to stand a few hours before filtering and the precipitate washed with saturated ammonium sulphate solution.

The protein fractions were suspended in salt solution and dialyzed in collodion sacs for four days in running water. After this time the sacs were suspended in changes of salt solution, the contents made up to the original volume of serum used, and placed in stoppered bottles.

Throughout the process, the solutions used were made up to 0.5 percent phenol concentration. In the case of the albumin precipitation, it was necessary to add a definite amount of 5 percent phenol to each lot of filtrate in order to counteract the diluting effect of the dissolved ammonium sulphate crystals. It was found that an additional 25 c.c. of phenol to each liter of albumin filtrate was sufficient to maintain a germicidal concentration when solid ammonium sulphate was added to the point of saturation. During filtration the funnels were kept covered and the filtrates collected in flasks to prevent undue change in concentration.

Test B.—A new lot of serum (250 c.c.) from the same stock (Serum 121) was fractionated without previous filtration by exactly the same method as that just outlined. The only variations to be noted are those in the volumes of the respective final filtrates obtained from precipitates and the final volumes of the separated fractions which in the test were made up to 500 c.c. with phenolized physiologic salt solution.

The amounts of saturated ammonium sulphate solution to be added in order to raise a solution to the proper concentration were calculated from the formula

$$x = v\,\frac{(c_2 - c_1)}{100 - c_2}$$

in which

x = number of cubic centimeters of saturated ammonium sulphate necessary to bring solution to required concentration.
v = number of cubic centimeters in original solution.
c_1 = percentage concentration (initial).
c_2 = percentage concentration (final, or desired).

QUANTITATIVE

Analyses were made of the serum to determine the percentage composition. Two methods were followed in this part of the work. The first method used was step for step like that used in the qualitative separation of the fractions to be used for injection (q.v.). The second was a modification of the Wiener[17] method.

Method 1.—Fifty and thirty-five cubic centimeters of test serum were precipitated respectively by half saturation with ammonium sulphate and further fractionation carried out as described in an earlier part of the paper. The precipitates were collected on weighed, small filter papers of very fine quality (dried

17. Ztschr. f. physiol. Chem., 1912, 74, p. 29.

in oven for one hour at 110-115 C., placed in desiccator for twenty minutes, and weighed). After thorough washing with the proper concentration of ammonium sulphate, each precipitate was placed in an electric oven at 110-115 C. for one and one-half hours in order to coagulate the proteins. Sulphates were removed by washing with boiling distilled water until tests with barium chlorid gave no further precipitate on the filtrate. The precipitates were again dried in the oven at 110-115 C. for an hour, placed in a desiccator for twenty minutes to one-half hour, and weighed rapidly. The results given below are for a test made in duplicate:

Fraction		Weight of Filter Paper in Gms.	Weight of Paper + Precipitate in Gms.	Weight of Precipitate in Gms.	Percentage Dry Weight	Average
Pseudoglobulin	A	0.4915	0.5320	0.0405	0.63	
Pseudoglobulin	B	0.5200	0.5430	0.0230	0.50	0.56
Euglobulin	A	0.5020	1.8160	1.3140	20.3	
Euglobulin	B	0.5270	1.4340	0.9070	20.0	20.15
Albumin	A	.....	.....	3.8750	59.8	
Albumin	B	.....	.....	2.5540	56.4	58.1

The percentage dry weight was calculated from the weighed residue obtained by evaporating a weighed amount of the same serum undergoing test. Five cubic centimeters of the serum were placed in a porcelain crucible and heated in an electric oven at 45 C. for fifteen hours, then placed for six hours in an electric oven at 110-115 C.

Weight of 5 c.c. serum = 4.9715 gm.
Weight of 5 c.c. serum (dried) = 0.6470 gm.

SUMMARY OF ANALYSES

	50 c.c. Serum	35 c.c. Serum
Percentage euglobulin.....	20.3	20.0
Percentage pseudoglobulin.....	0.63	0.5
Percentage albumin	59.8	56.4
Percentage of total protein.....	80.73	76.9

The total protein in the blood plasma of the pig was found by Lewinsky[18] to be 80.5 percent of the whole.

Method 2.—Wiener[19] has stated that the weight of protein precipitated from greatly diluted serum by one-half saturation with ammonium sulphate is always less than that precipitated from undiluted serum. He accepts the lower value as the correct one since the precipitate from undiluted serum carries with it protein belonging to other more soluble fractions and since the precipitate from diluted serum is quite insoluble in ammonium sulphate of the corresponding concentration. The precipitate from undiluted serum, furthermore, cannot be freed entirely from foreign proteins by washing with ammonium sulphate solution.

Twenty cubic centimeters of serum from the same lot were diluted to a volume of 400 c.c. with physiologic salt solution. Instead of fractionating the globulin, the total globulin was precipitated by means of half saturation with ammonium sulphate, as a check upon the method used in separating the protein

18. Pflüger's Arch. f. gesammt. Physiol. d. Mensch. u. Thiere, 1903, 100, p. 611.
19. Ztschr. f. physiol. Chem., 1910, 74, p. 29.

fractions for injection (q. v.). The precipitate was washed with 50 percent ammonium sulphate until protein-free and dried for one hour in the electric oven at 110-115 C. to coagulate the protein. The precipitate was washed with boiling distilled water until free from sulphate, dried in the oven, and weighed.

Weight of Filter Paper	Weight of Paper and Precipitate	Weight of Precipitate	Percentage of Original Protein
1.6330 gm.	2.1764 gm.	0.5434 gm.	21.0

From this result, it will be seen that the total euglobulin obtained by adding the euglobulin and pseudoglobulin fractions from undiluted serum was about the same as that obtained from diluted serum with one precipitation (see results of analysis). At first glance, this is not in harmony with Wiener's work, if we may accept this one analysis as a criterion. It is to be noted however that in the first method of separation used in this paper, the globulin was further split up and "purified," so that the fractions were free of more soluble proteins. The total globulin thus obtained is not comparable to a globulin precipitate which might be brought out of solution by a single precipitation by means of half saturation with ammonium sulphate. We have in reality an actual dilution and hence the results are essentially in accord with Wiener's findings.

IMMUNIZATION WITH PROTEIN FRACTIONS FROM HCG-CHOLERA SERUM

Six hogs were injected with euglobulin, pseudoglobulin, and albumin fractions, respectively, plus virus. Each animal received 2 c.c. of virus simultaneously with the serum. Intramuscular injections were made.

No. of Hog	Weight lbs.	Virus c.c.	Serum c.c.	Fraction *	Result
858	70	2	25	Whole serum	Well.
1606	80	2	25	Whole serum	Well.
1967	70	2	50	Euglobulin	Died. Bacillus suisepticus found in pure culture.
1156	65	2	50	Euglobulin	Well.
1481	60	2	50	Pseudoglobulin	Well.
982	60	2	50	Pseudoglobulin	Well.
1592	60	2	..	.....	Bled. Typical cholera lesions.
1164	60	2	..	.....	Bled. Typical cholera lesions.
900	60	2	50	Albumin	No protection. Killed. Typical cholera lesions. + + +
901	70	2	50	Albumin	No protection. Killed. Typical cholera lesions. + +

* The fractions were made up to double the volume of original serum.

A review of this result shows that both the euglobulin and pseudoglobulin fractions of the serum possess marked protective value. In other words, the globulin fractions, as a whole, contain the antibodies. The animals, with exception of the "virus" and "albumin" hogs, and one hog treated with euglobulin, were never off feed. The last named was chronically ill and died after two weeks. Some protection was given by the fraction injected, but secondary complications appeared to have set in. Postmortem lesions were typical of cholera and Bacillus suisepticus was found in all organs. Intraperitoneal doses of 0.5 c.c. of an agar slant emulsion made from these cultures killed guinea-pigs in two to three days.

The protective value of these fractions, taken together, is about five pounds for each cubic centimeter of serum. Comparison with the potency of whole serum renders this figure somewhat inconsistent. The whole serum, as shown in Tables 1 and 2, gave about three pounds protection for each cubic centimeter. However, since there were but slight, if any, untoward symptoms in these test animals, it is very likely that a smaller amount of serum would have protected as well as the fractional serum. In that event, the protective value, expressed in pounds body weight, would be about the same. Moreover, some of the animals treated with the serum fractions ran close to the margin, as may be seen from the temperature chart. Administration of somewhat larger doses would of necessity have lowered the figures for protective value per pound and thus have approximated more nearly the value in terms of unfractionated serum. Because of a scarcity of material, it was not possible to run detailed quantitative experiments. It may be of interest to determine by varying doses whether or not the fractions possess different degrees of protective power. The quantitative determinations made on the serum point to the fact that the pseudoglobulin fraction is considerably more potent than the euglobulin. The latter constitutes about 20 percent of the total protein whereas the former represents but 0.5 percent, being thus forty times more effective. With such a small series, however, these figures must not be regarded as conclusive, even if we were to overlook the very important factor of idiosyncrasies in individual animals. Differences in antibody content may be due to a number of factors. As has been intimated in the chemical separation, a loss may occur in the lower fraction due to its solubility in the higher concentration of ammonium sulphate. Any relative differences in the distri-

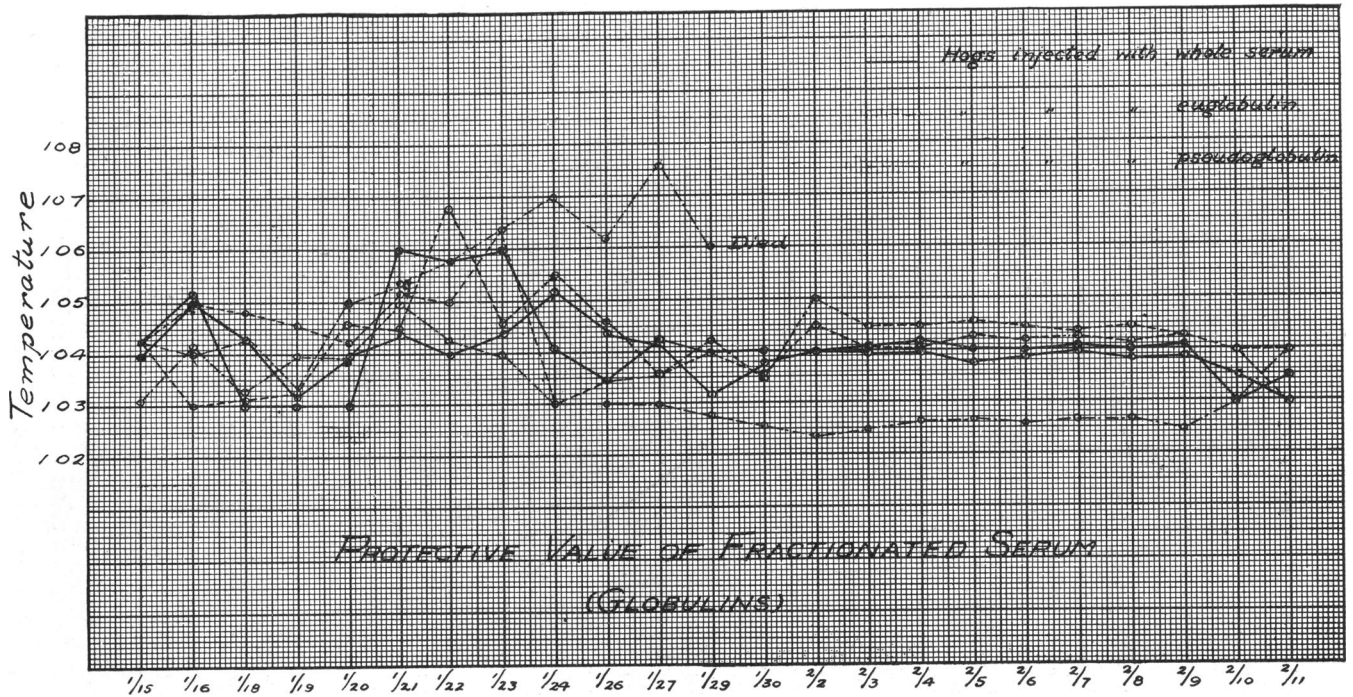

Chart 1.—The globulin fraction here shows protection. The animals with one exception were protected. This one at autopsy, as noted elsewhere, showed complications.

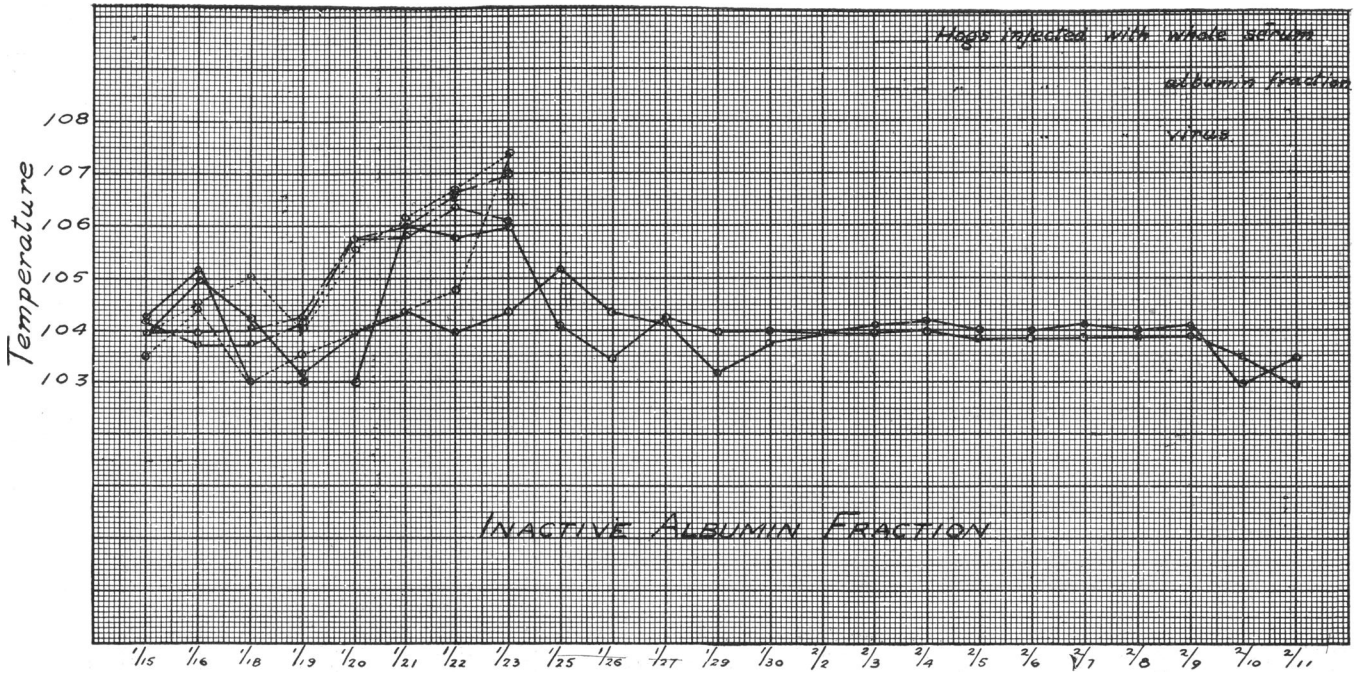

Chart 2.—The albumin fraction does not exert any protection. The temperature curve follows closely that observed in the case of hogs treated with virus alone.

bution of antibodies should be more pronounced for the lower fraction. This has been shown by Gibson and Collins[20] in their work on diphtheria antitoxin. It is not likely that this phase enters here in so striking a manner, since the material injected was not in concentrated form and since the possible inclusion of a lower fraction in the higher would not be sufficiently great to alter the protective value to any extent.

The albumin fraction is entirely inactive. On the seventh day, the animals ran a high temperature and were very sick. Death was imminent on the eighth day. A comparison between the temperature curves of these animals and those of the controls (virus alone) will show a striking similarity.

The charts here given show graphically the relative value of the serum fractions. Chart 1 represents the effectiveness of the split globulins as compared with the unfractionated serum. In Chart 2 are given the results obtained with albumin separated from hog-cholera antiserum.

SUMMARY AND CONCLUSIONS

Hog-cholera serum can be split up by chemical means into an actively protecting globulin fraction and an inactive albumin fraction.

Precipitation of serum proteins by means of ammonium sulphate is practically possible for hog-cholera serum. The bulk of the serum, being inactive albumin, may be dispensed with.

Concentration for practical purposes may be effected (1) by precipitating the euglobulins from diluted serum, by means of 33⅓ percent saturation with ammonium sulphate solution, filtering, making the filtrate up to 50 percent concentration with ammonium sulphate solution, filtering, and after dialyzing the precipitate in running water, dissolving it in the smallest volume of salt solution; (2) by precipitating the diluted serum (diluted 10-15 times) by one-half saturation with ammonium sulphate (saturated solution), filtering, dialyzing the precipitate, and treating as in (1). Since both globulin constituents are protective, this method would prove more economical and simpler.

Euglobulin represents from 20-21 percent of the total serum protein, pseudoglobulin 0.5 percent, and albumin about 80 percent.

20. Jour. Biol. Chem., 1907, 3, p. 233.